REMARKS

Claims 4 and 27-36 are under consideration.

Rejections under 35 USC § 103

Claims 4, 27-31, and 33-36 are rejected under § 103(a) as allegedly unpatentable over Balasubramanisn (WO 01/57248; published 9/2001) as evidenced by Cheeseman [United States Patent Number (USPN) 5,302,509; issued 1994] and in view of Lackey et al. (USPN 5,652,126) and Hong et al. (USPN 5,747,298; issued 1998). In view of the arguments presented herein, this rejection is respectfully traversed.

Responsive thereto, Applicant asserts that the combined references do not teach or suggest all elements of the claimed invention. The claims require, *inter alia*, (d) performing a second round of sequencing of each of the immobilised single-stranded template nucleic acid molecules regenerated in step (c) by synthesising a second complementary copy of each of the template sequences, wherein said synthesizing involves repeated cycles of incorporating a single nucleotide into the second complementary copy and detecting incorporation of the single nucleotide into the second complementary copy on the array, thereby generating a sequence of the second complementary copy; and (e) comparing the sequence of the first complementary copy detected in step (b) to the sequence of the second complementary copy detected in step (d) for each of the immobilized single-stranded template nucleic acid molecules to confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules. As detailed herein below, the combined disclosures of Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. fail to teach or suggest performing a second round of sequencing of each of the immobilised single-stranded template nucleic acid molecules regenerated in step (c) and comparing the sequence of the first complementary copy detected in step (b) to the sequence of the second complementary copy detected in step (d) for each of the immobilized single-stranded template nucleic acid molecules to confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules. Applicant, therefore, asserts that the combined disclosures of Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. fail to teach or

suggest all of the elements of the instant claims and thus, fail to adversely impact the patentability of the claimed method.

The Office Action acknowledges that Balasubramanisn et al. do not teach a method further comprising removing the complementary copy of each of the template sequences from the array thereby regenerating the immobilized single stranded template molecules on the array (claims 4, 35, and 36 (step c)); performing a second round of sequencing of each of the immobilized single stranded template nucleic acid molecules regenerated in step (c) by synthesizing a second complementary copy of each of the template sequences, wherein said synthesizing involves repeated cycles of incorporating a single nucleotide into the second complementary copy and detecting incorporation of the single nucleotide into the second complementary copy on the array, thereby generating a sequence of the second complementary copy (claims 4, 35, and 36 (step d)); and comparing the sequence of the first complementary copy detected in step (b) to the sequence of the second complementary copy detected step (d) for each of the immobilized single-stranded template nucleic acid molecules to confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules (claims 4, 35, and 36 (step e)). The Office Action also acknowledges that Balasubramanisn et al. do not teach a method wherein the double stranded anchor comprises a recognition site for a restriction endonuclease (claim 29) or a method wherein the comparing step reduces random sequencing errors of the template sequences arising from the first round of sequencing (claim 34).

The Office Action, however, relies on Lackey et al. for teaching a method that comprises synthesizing a complementary copy of a nucleic acid sequence using a primer and a template sequence. Lackey et al. is maintained as further teaching that in instances where a DNA/primer template with a single 3' ribonucleotide is used, cleavage at the ribonucleotide residue, followed by separation and purification of the oligonucleotide product, results in a fully regenerated and reusable primer/template (column 13, lines 26-31). Lackey et al. is also viewed as teaching that cleavage may be performed using a site specific restriction endonuclease, alkaline hydrolysis, or an endonuclease such as RNase (column 12, lines 42-47). The Office Action concludes, therefore, that Lackey et al. teach a method comprising removing the complementary copy of a

template sequence thereby regenerating the template and a method wherein the primer has a recognition site for a restriction endonuclease.

The Office Action does, however, acknowledge that Lackey is not directed to sequencing. Applicant agrees. As evidenced throughout the reference, the methods of Lackey et al. are directed to the generation of pure populations of like oligonucleotides for use as probes. The reference is silent with respect to sequencing. In view of the fact that the sequences of the templates from which the oligonucleotides are made are already known, there is no need or motivation for a skilled practitioner to consider sequencing in the context of this reference.

The Office Action relies on Hong et al. for teaching that researchers in the field of DNA sequencing often have to use several approaches to confirm their findings in order to avoid being misled by potentially erroneous sequence data. Hong et al. is also viewed as teaching that sometimes researchers rely on repeating the same sequencing experiment with different DNA polymerases, or performing another sequencing reaction with the template that is complementary to the first single-stranded DNA template, and comparing the results for possible discrepancies (column 2, lines 47-55). The Office Action concludes that Hong et al., therefore, allegedly teach performing a second round of sequencing on the same template (*emphasis added*) used in the first round of sequencing and comparing the sequence of the first complementary copy to the sequence of the second complementary copy in order to confirm sequence data.

Responsive thereto, Applicant asserts that the method of Hong et al. does not perform a second round of sequencing on the same template used in the first round of sequencing. In contrast, the method of Hong et al. calls for performing a round of sequencing on a first pool of template nucleic acid molecules and a round of sequencing on a second pool of template nucleic acid molecules, and comparing the sequencing results generated for the first and second pools for possible discrepancies. It is noteworthy that the first and second pools of template nucleic acid molecules are independent pools. In keeping with the teaching of Hong et al., it is apparent that repeating a sequencing experiment is achieved either by using a different DNA polymerase in the second round of sequencing performed on the second independent pool of template nucleic acid molecules than that used for sequencing the first pool of template nucleic acids, or by performing

a second sequencing reaction with templates that are complementary to the first single-stranded DNA templates, and comparing the results of the first and second rounds of sequencing for possible discrepancies. Neither of these scenarios involves resequencing the same template pool sequenced in the first round of sequencing. The former of these scenarios is performed using two independent and presumably identical pools of template nucleic acid molecules, whereas the latter scenario is performed with two independent and non-identical pools of template nucleic acid molecules. This stands in marked contrast to the method of the present invention, wherein a second round of sequencing is performed such that the exact same collection of immobilised single-stranded template nucleic acid molecules is resequenced in a second round of sequencing.

That being the case, performing a second round of sequencing as taught by Hong et al. is not equivalent to performing a second round of sequencing of each of the immobilised single-stranded template nucleic acid molecules regenerated in step (c) as recited in step (d) of the instant claims. In that the Office Action recognizes that Hong et al. is the only cited reference that teaches "performing a second round of sequencing", Applicant asserts that the combined teachings of the Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. fail to teach or suggest step (d) of the instant claims.

Moreover, the deficiencies of these references with respect to step (d) ultimately lead to and underscore their shortcomings with respect to step (e) of the instant claims. Step (e) calls for comparing the sequence of the first complementary copy detected in step (b) to the sequence of the second complementary copy detected in step (d) for each of the immobilized single-stranded template nucleic acid molecules to confirm sequencing data of each of the immobilized single-stranded template nucleic acid molecules. As described in Hong et al. and discussed herein above, "performing a second round of sequencing" invariably involves sequencing a second independent pool of template nucleic acid molecules, which is presumed to be identical or chosen to be non-identical to that of the first pool of template nucleic acid molecules. Under either model, the potential for comparing a first sequencing read of a particular template nucleic acid molecule to a second sequencing read of the regenerated particular template nucleic acid molecule is nil. In view of the above, "performing a second round of sequencing" in accordance

with the teaching of Hong et al. does not teach step (e) and cannot achieve the objective of step (e) of the instant claims. In that Hong et al. is the only cited reference that teaches "performing a second round of sequencing", Applicant asserts that the combined teachings of the Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. fail to teach or suggest step (e) of the instant claims.

Further to this point, Applicant asserts that the method of Hong et al. lacks the resolution of the instant method to compare first and second sequencing reads on the level of an individual template nucleic acid molecule. The method of Hong et al. calls for performing a first round of sequencing on a first pool of template nucleic acid molecules and a second round of sequencing on a second pool of template nucleic acid molecules, and comparing the sequencing results generated for the first and second pools for possible discrepancies. Gel-based sequencing methods such as those described by Hong et al. cannot be used for comparison of first and second sequencing reads for any individual template nucleic acid molecule in a pool of template nucleic acid molecules. In contrast, the method is limited to providing an average of sequencing reads for several molecules, which can under some circumstances have the same or overlapping sequence.

Briefly, the method of Hong et al. calls for performing the standard Sanger protocol for single-strand DNA (ssDNA) sequencing, using the Bst DNA polymerase of the invention, in a centrifuge tube. Accordingly, sequencing ladders produced thereby could be generated from any one of a plurality of ssDNA templates in the centrifuge tube and there is no way to determine which sequencing ladder is an extension product of a particular ssDNA template in the reaction mix. The pool of sequencing ladders so generated is then subjected to electrophoresis in a denaturing high resolution polyacrylamide gel. Thus, sequencing in the context of Hong et al. is only applicable to evaluating a population en masse, but is not capable of yielding any information that can be ascribed to a particular template molecule in the population.

In light of the above, Applicant asserts that the combined teachings of Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. fail to teach steps (d) and (e) of the instant claims. Accordingly, Applicant asserts that the Office Action has failed to establish a *prima*

facie case of obviousness.

Turning next to the potential for motivation to combine the teachings of the cited references, the Office Action appears to view Hong et al. as providing motivation to confirm sequencing data. More particularly, the Office Action looks to Hong et al. for allegedly providing motivation to modify the method of Balasubramanisn et al. by removing the first complementary strand to regenerate the template molecule (as allegedly suggested by Lackey et al.), resequencing the regenerated template molecule (as allegedly suggested by Hong et al.), and comparing the sequence of the first complementary copy and the second complementary copy to confirm sequence data (as allegedly suggested by Hong et al.). The Office Action maintains that Hong et al. teaches that researchers in the field of DNA sequencing often have to use several approaches to confirm their findings in order to avoid being misled by potentially erroneous sequence data. At the outset, Applicant respectfully disagrees with the Office Action's assertions regarding the teaching of Hong et al. This reference does not teach or suggest resequencing regenerated template molecules. Hong et al. fail to teach or suggest regenerated templates. In all manifestations, Hong et al. teach performing a second round of sequencing using a second, independent pool of template nucleic acid molecules. Clarification of the record on this point is thus necessitated.

Moreover, the Office Action has failed to provide a rationale to support the contention that it would allegedly have been obvious for an ordinarily skilled practitioner to combine the methods of Balasubramanisn et al., Cheeseman, Lackey et al., and Hong et al. to arrive at the present invention. More particularly, there is no rationale provided in the Office Action to explain why an ordinarily skilled artisan would modify the method of Balasubramanian et al. to add the step of removing the first complementary strand to regenerate the template molecule as allegedly taught by Lackey et al. The Office Action erroneously states that Hong et al. teach "resequencing the regenerated template molecule". There is no factual basis to support the Office Action's position in this regard. Absent such teaching, Hong et al. fails to provide any stimulus that would motivate an ordinarily skilled practitioner to combine the teaching of Balasubramanisn et al. with those of Lackey et al. As discussed in greater detail below, the

teaching of Hong et al. would at best motivate an ordinarily skilled practitioner to perform a second round of sequencing as taught by Hong et al.

The Office Action acknowledges that Hong et al. is the only cited reference that teaches "performing a second round of sequencing". Accordingly, Applicant asserts that should one skilled in the art have been motivated to modify the teaching of Balasubraminism et al. to include aspects of Hong et al. directed to confirming sequencing data, such a practitioner would have been guided by the teaching of Hong et al. with respect to "performing a second round of sequencing". Following the first scenario described by Hong et al., which involves sequencing a second independent pool of template nucleic acid molecules using a different DNA polymerase, a skilled practitioner might have prepared two separate arrays using essentially identical pools of template nucleic acid molecules and performed sequencing reactions in accordance with Balasubraminism et al., wherein a different DNA polymerase is used to sequence each of the arrays. In so doing, the practitioner would "repeat the sequencing experiment" in accordance with Hong et al. Following the alternative scenario of Hong et al., such a practitioner might have prepared two separate arrays, one of which has a first pool of template nucleic acid molecules, whereas the other has a second pool of templates that is complementary to the first pool of template nucleic acid molecules, and performed sequencing reactions in accordance with Balasubraminism et al. on both arrays, so as to "repeat the sequencing experiment" as taught by Hong et al. In light of the above, the motivation to "repeat the sequencing experiment" as allegedly conferred by Hong et al. might lead an ordinarily skilled practitioner to undertake one of the above scenarios that combines the teachings of Balasubraminism et al. and Hong et al., but would not lead to a realization of the instant method.

The Examiner is respectfully reminded that while KSR "counsels against applying the [TSM test] as a rigid and mandatory formula... it remains necessary to show 'some articulated reasoning with some rational underpinning to support the legal conclusion on obviousness." *Aventis Pharma v. Lupin* (citing *KSR v Teleflex*). *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007), moreover, requires that an Examiner provide "some articulated reasoning with some rationale underpinning to support the legal conclusion of obviousness." 127 S.Ct. at 1741. An

Examiner must "identify a reason that would have prompted a person of ordinary skill in the relevant field to **combine the elements in the way the claimed new invention does**," Id. Under the present circumstances, the Examiner minimally has not identified a reason that would have prompted an ordinarily skilled practitioner to modify the alleged combined teachings of Balasubraminism et al. and Hong et al. to incorporate aspects of Lackey et al. In light of the above, the Office Action has failed to establish a *prima facie* case of obviousness.

In view of the above, Applicant asserts that the combined teachings of Balasubramanian et al., Cheeseman, Lackey et al., and Hong et al. fail to teach several recited elements of the claims and the alleged motivation to combine the teachings of these references to arrive at the claimed method is not properly substantiated. That being the case, the Examiner is respectfully requested to reconsider the validity of the rejection of claims 4, 27-31, and 33-36 under 35 U.S.C. §103 and withdraw the rejection.

Claim 32 is rejected under § 103(a) as allegedly unpatentable over Balasubramanisn (WO 01/57248; published 9/2001) as evidenced by Cheeseman [United States Patent Number (USPN) 5,302,509; issued 1994] in view of Lackey et al. (USPN 5,652,126) and Hong et al. (USPN 5,747,298; issued 1998) as applied to claims 4 and 31 above and further in view of Barnes (WO 01/57249; published 8/2001). In view of the arguments presented herein, this rejection is respectfully traversed.

The Office Action acknowledges that the combined teachings of Balasubramanian et al. (as evidenced by Cheeseman), Lackey et al., and Hong et al. do not teach a method wherein the fluorescently labeled nucleotides are detected using a microscope with total internal reflection based imaging. The Office Action relies on Barnes for teaching that using total internal reflection fluorescent microscopy makes it possible to achieve wide field imaging with single polymer sensitivity. Statements pertaining to the deficiencies of the combined teachings of Balasubramanian et al., Cheeseman, Lackey et al., and Hong et al. are set forth in detail above and the aforementioned statements are incorporated herein by reference in their entireties. At the very least, the combined teachings of Balasubramanian et al., Cheeseman, Lackey et al., and Hong et al. fail to teach or suggest all of the recited elements of the instant claims. The teachings

of Barnes fail to compensate for the aforementioned defects of these references in combination. Moreover, Barnes fails to provide motivation to combine the teachings of these references to arrive at the present invention. That being the case, Applicant maintains that the combined teachings of Balasubramanian et al., Cheeseman et al., Lackey et al., Hong et al., and Barnes would not lead an ordinarily skilled practitioner to arrive at the present invention.

In view of the above, the Examiner is respectfully requested to reconsider and withdraw the rejection of claim 32 under 35 U.S.C. §103.

Fees

No additional fees are believed to be necessitated by this amendment. However, should this be an error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment or to credit any overpayment.

Conclusion

It is submitted, therefore, that the claims are in condition for allowance. No new matter has been introduced. Allowance of all claims at an early date is solicited. In the event that there are any questions concerning this amendment, or application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

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